

We performed some experiments with HPLC for the iGEM Paris Bettencourt team, in order to plot the capability of *Pseudomonas Putida* to biodegrade Anthocyanins, including Malvidin.

The experiments were done by means of preparing several solutions with concentration that come from 25 mg/L to 1 mg/L of Anthocyanins, see **Figure A to C** . Once prepared 5 mL of each solution (A to F) in a Falcon tube of 50 mL. An inoculum of 100 uL of an O/N culture of *Pseudomonas putida* KT2440 was introduced in the solution. During 5-6 days. Samples were taken every day in order to plot a graph in which to follow the rate of degradation of Anthocyanins, including Malvidin between them.

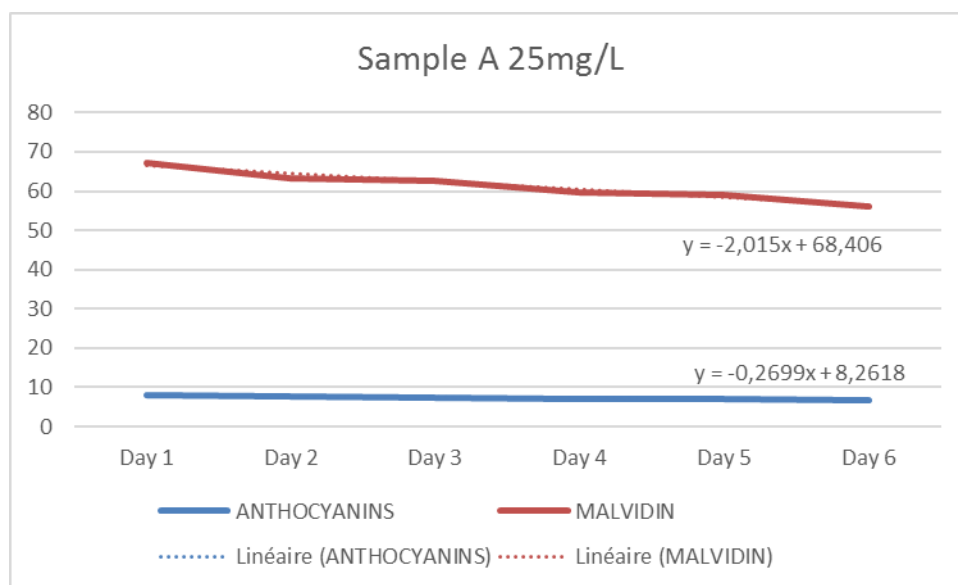


Figure A: Degradation plot of Sample A at 25mg/L

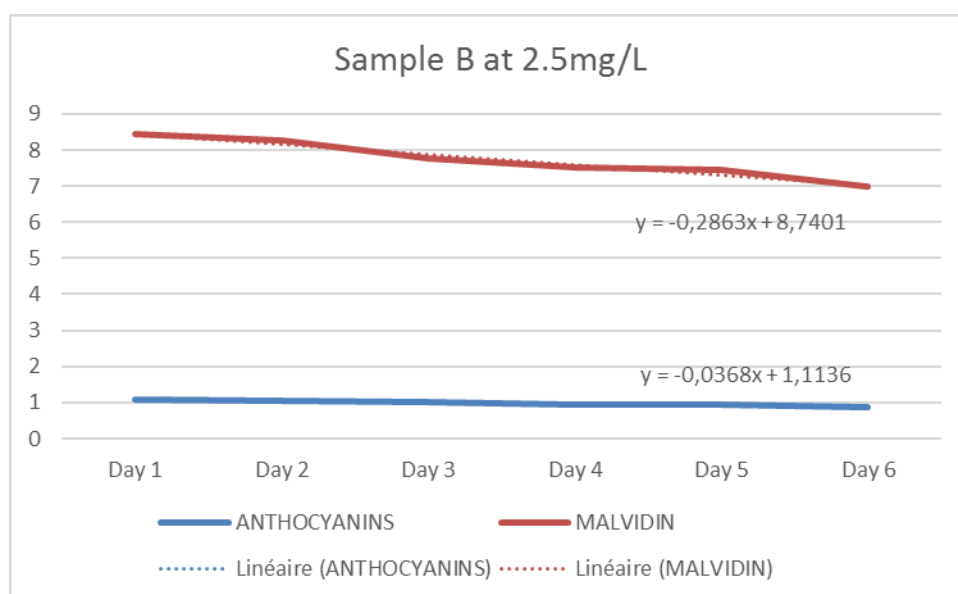


Figure B: Degradation plot of Sample B at 2.5mg/L

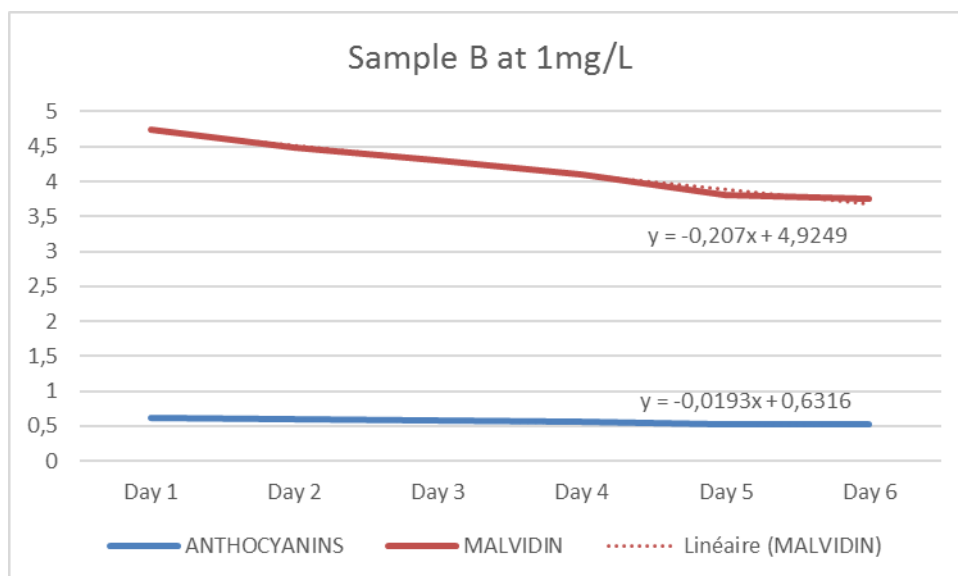


Figure C: Degradation plot of Sample B at 1mg/L

The following Figure 1 and Figure 2 contains the curves of degradation of all the Anthocyanin present in the solution, as well as the detected Malvidin like-compounds.

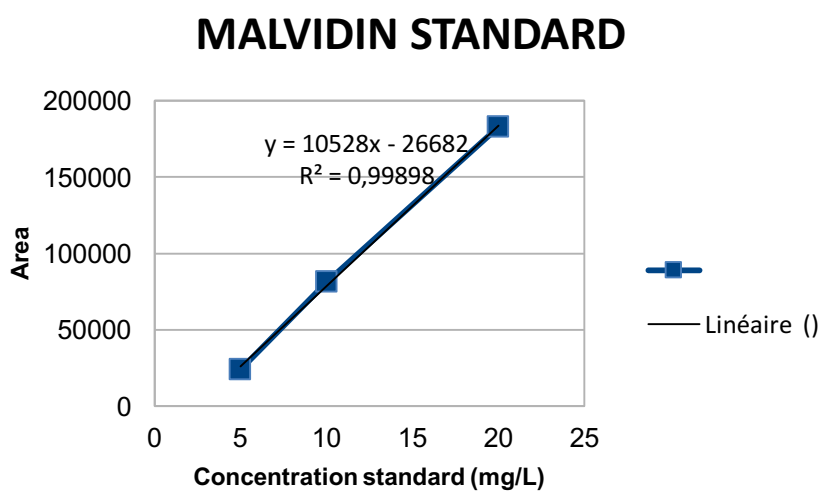


Figure 1: Malvidin standard degradation

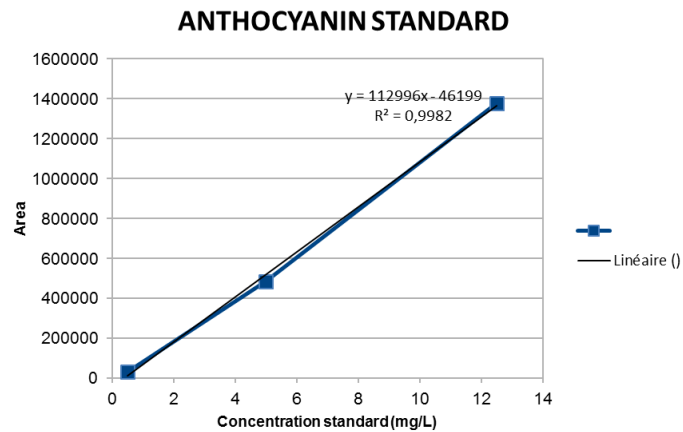


Figure 2: Anthocyanin standard degradation

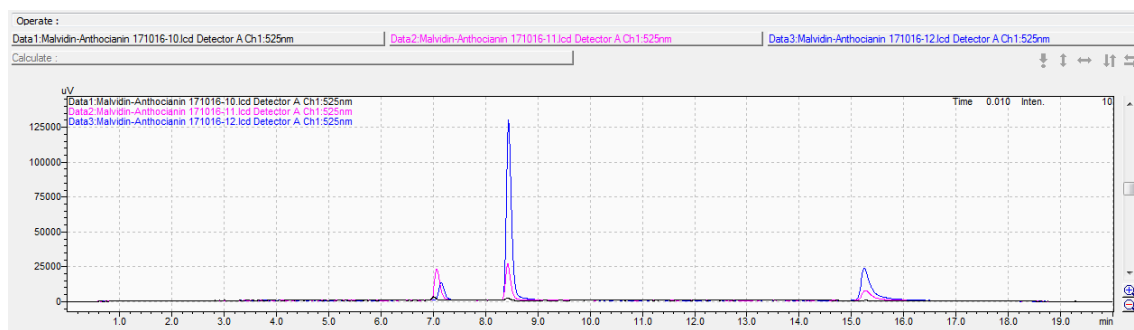


Figure 3A : This image shows the elution times for the 3 types of anthocyanin-like compounds detected on the HPLC for the sample of anthocyanin used for the biodegradation experiments. peak 1 is named Anthocyanin 1, peak 2 is named Malvidin-like compounds and peak 3 is named Anthocyanin 3. The elution times are 7, 8.5 and 15.3 minutes, respectively.

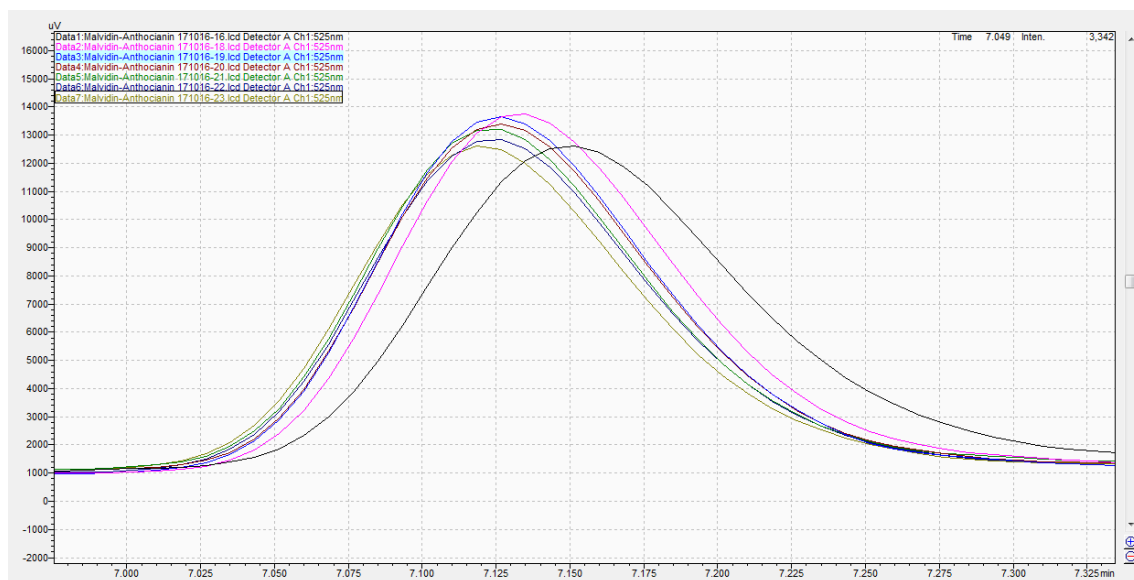


Figure 3B. This graph shows the pattern of degradation of Anthocyanin-1-like compounds in a sample of 25 mg/L of Anthocyanins over time. Data1 corresponds to the sample run to detect the elution time (7 min) and from Data2 to Data7, are the peaks corresponding to each day of sampling. It can be appreciated that the compound shows lower concentrations over time.

Black=Elution time sample; Pink=day 1, Blue=day2, Red=day3, Green= day4, Dark-blue=day5, Yellow=day6

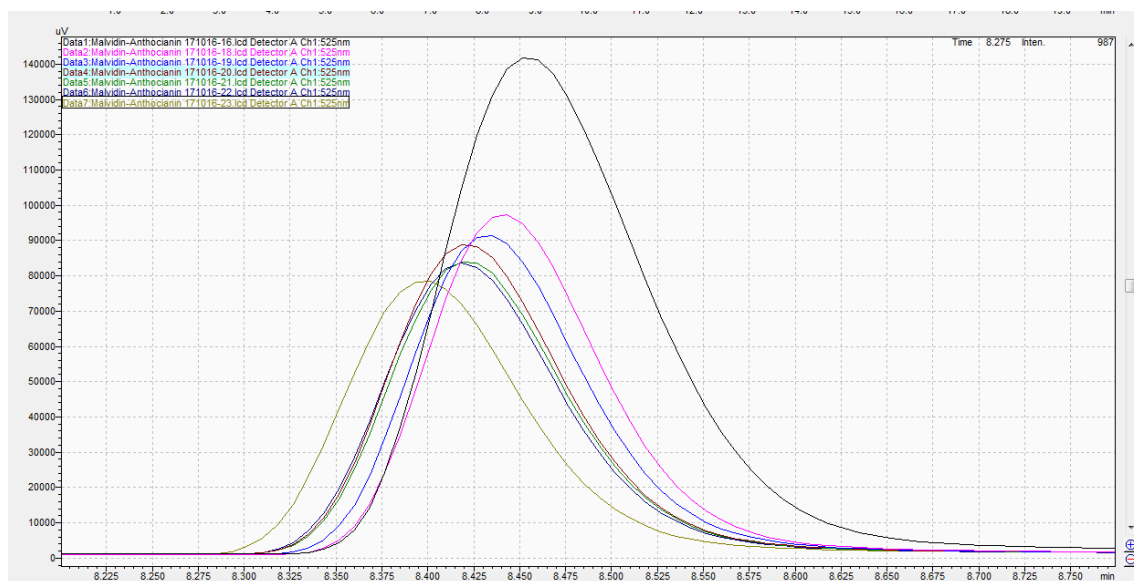


Figure 3C. This graph shows the pattern of degradation of Malvidin-like compounds in a sample of 25 mg/L of Anthocyanins over time. Black peak corresponds to the sample run to detect the elution time (8.5 min) and from pink to yellow peaks, are the data corresponding to each day of sampling. It can be appreciated that the compound shows lower concentrations over time.

Black=Elution time sample; Pink=day 1, Blue=day2, Red=day3, Green= day4, Dark-blue=day5, Yellow=day6

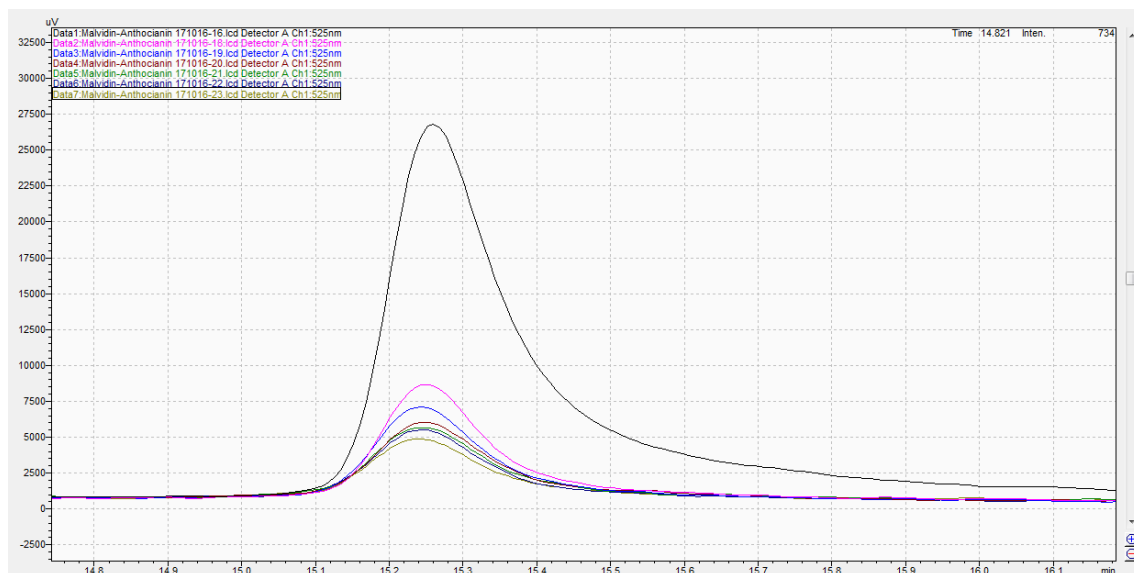


Figure 3D. This graph shows the pattern of degradation of Anthocyanin-1-like compounds in a sample of 25 mg/L of Anthocyanins over time. The biggest peak corresponds to the sample run to detect the elution time (15.3 min) and from pink to yellow peaks, are the peaks corresponding to each day of sampling. It can be appreciated that the compound shows lower concentrations over time. Black=Elution time sample; Pink=day 1, Blue=day2, Red=day3, Green= day4, Dark-blue=day5, Yellow=day6

The reason why the peak for Malvidin-like, compounds is much bigger than expected is because, in order to detect Malvidins alone, an independent standard with a purified version of it was used for detection. Because of that, we hypothesize that since in the anthocyanin prepared at different dilutions contain several compounds with similar elution times than the Malvidin, those are included in the peak.